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# Beltsville Human Nutrition Research Center



1992

United States Department of Agriculture Agricultural Research Service

Beltsville, Maryland 20705



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# United States Department of Agriculture Agricultural Research Service Beltsville Human Nutrition Research Center Beltsville, Maryland 20705

Mission: To (1) conduct research relevant to human requirements for energy, protein, carbohydrates, lipids, vitamins and minerals and their bioavailability from commonly eaten foods which will assure optimal function throughout the life cycle; (2) develop dietary strategies which can lead to postponement of the onset of nutritionally-related debilitating diseases. In carrying out this twofold mission small laboratory animal models are developed and utilized for determination of design and performance of human studies. Animal studies are used to establish new hypotheses, test existing ones and to clarify basic metabolic function of nutrients. Controlled human dietary-metabolic studies are used as the experimental tests upon which can be developed dietary strategies for a healthy Nation and guidance for improving the nutritional quality of food crops and animals.



# Office of Center Director Beltsville Human Nutrition Research Center Beltsville, Maryland 20705

<u>Mission</u>: The Office of Center Director provides for coordination, evaluation and safety of all nutritional studies involving human subjects and for the rapid application to human studies of knowledge concerning nutrient bioavailability and interactions, metabolic mechanisms of action and nutrient requirements which were developed through studies using animal models.

Dr. Walter Mertz Director Room 223, Building 308 Beltsville, Maryland 20705 301/504-8157

Ms. Priscilla D. Steele Chief Dietitian Room 326, Building 308 Beltsville, Maryland 20705 301/504-8411 Provides leadership to the Center. Studies health-related problems relating to nutriture of trace elements.

Provides leadership to the Human Study Facility.



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## Carbohydrate Nutrition Laboratory Beltsville Human Nutrition Research Center Beltsville, Maryland 20705

Mission: (1) determine the effects of utilizable carbohydrates such as sucrose, fructose and starch on risk factors associated with human disease and investigate the mechanisms for their differential effects; (2) determine the effects of dietary fibers on nutrient bioavailability and to investigate the mechanisms underlying these effects; (3) determine interactions that occur between dietary carbohydrates and various minerals; and (4) determine the effects of these interactions on population groups with different genetic predisposition (e.g., carbohydrate sensitivity) in order to identify those individuals at particular risk. The information to be derived from these studies will enable the laboratory to attain its technical objective, the establishment of optimal levels for carbohydrate intake by humans and the forms that best meet these requirements, thus improving the health and quality of life in the adult and aging population.

Dr. Judith Hallfrisch Research Leader Supervisory Research Chemist Room 323, Building 307 Beltsville, Maryland 20705 301/504-8396

Dr. Kay M. Behall Research Chemist Room 305, Building 307 Beltsville, Maryland 20705 301/504-8682

Dr. Sam J. Bhathena Research Chemist Room 324, Building 307 Beltsville, Maryland 20705 301/504-8422 Provides leadership to the laboratory. Studies: (1) the beneficial and detrimental effects of recent changes in the U.S. carbohydrate supply on health and performance in humans and animals; (2) mechanisms of interactions between carbohydrates and minerals; (3) differential sex and age effects of these interactions; (4) the relation of carbohydrate nutrition to heart disease, diabetes and cancer.

Investigates effects of different sources of starch or fiber on metabolic risk factors associated with human diseases. Studies the effects of chemically-defined dietary fiber on metabolic and physiological processes associated with heart diseases, diabetes, bowel function and mineral balance in humans.

Studies the effects of dietary carbohydrates on hormones involved in carbohydrate metabolism and on erythrocyte and tissue receptors of hormones such as insulin and glucagon in normal and carbohydrate-sensitive subjects hormone and opiate receptors in obese and spontaneously hypertensive rats. Role of opiates in diabetes, obesity, food intake, and food preferences experimental animals and humans. Role of opiates and neuropeptides in copper deficiency. Modulation of peptides and neuropeptides by copper and zinc.



Dr. Otho E. Michaelis IV Research Biologist Room 315, Building 307 Beltsville, Maryland 20705 301/504-8417

Dr. Bela Szepesi Research Chemist Room 313, Building 307 Beltsville, Maryland 20705 301/504-8489

Dr. David L. Trout Research Nutritionist Room 328, Building 307 Beltsville, Maryland 20705 301/504-8386 Studies the effects of feeding various carbohydrates to experimental animals with specific genetic predisposition toward obesity, hypertension, glucose intolerance, hyperlipidemia and how diet, sex, age and genetics interact to produce metabolic and structural defects.

Studies basic mechanisms by which dietary carbohydrates differentially affect the expression of genetic tendency for obesity, diabetes and degenerative diseases. Examines the functioning of metabolic controls, the alterations in these controls in various rat models of degenerative diseases and the effect and interaction of dietary carbohydrates with the above factors.

Studies metabolic and gastrointestinal responses to different sources of fiber and starches. Examines health consequences of bacterial fermentation of food residues in the lower digestive tract.



#### RECENT RESEARCH ACCOMPLISHMENTS

## Amylose Intake On Metabolic Parameters

The long-term effect of the type of starch in the diet was investigated in 24 men. Products containing cornstarch with 70% of the starch in the form of amylopectin (standard cornstarch) or amylose were each consumed for 14 weeks. Half of the subjects had elevated insulin response (HI). Results indicate that insulin levels of the HI were significantly lower after amylose when compared to amylopectin. Insulin response area was significantly lower in all subjects consuming amylose when compared to amylopectin with the greatest decrease occurring in HI. Glucose response to a starch load was similar in HI and control subjects and did not vary with the starch. Following a meal, triglyceride levels were significantly lower when subjects consumed amylose compared to amylopectin. Fasting triglycerides decreased significantly with time on diet. Cholesterol was significantly lower after amylose when compared to amylopectin after 4 weeks on each starch with similar reductions thereafter. Results indicate that chronic consumption of high amylose foods appears to normalize the insulin response of HI men. Increased starch consumption especially in the form of amylose helped decrease blood lipids, a factor associated with atherogenesis in humans.

# Diabetic SHR/N-cp Rats Devolop Inner Ear Neuropathy Associated With Human Diabetes

In collaboration with the Department of Otolaryngology, University of North Carolina, School of Medicine, it was demonstrated that diabetic SHR/N-cp rats fed a high carbohydrate diet develop neuropathy of the inner ear which is similar to that reported in human diabetics. Alterations observed included significant loss of outer hair cells, capillary basement membrane thickening and ganglion loss. These alterations were not observed in nondiabetic control animals.

# Insulin Secretory Patterns are Altered in Obesity and Diabetes

Adult onset diabetes and obesity are characterized by high blood glucose levels in the presence of hyperinsulinemia. We have observed three separate defects in diabetic rats namely 1) paradoxically high insulin secretion at low glucose level, 2) secretion of insulin in response to arginine in the absence of glucose, and 3) impaired response of insulin secretion to high glucose. In obese rats only first 2 defects are present. These defects can be reversed by prolonged fasting which reduces blood glucose level. Conversely, infusing normal rats with glucose for 48 hours induced these defects. Thus, our study shows that high blood sugar levels in diabetes reversibly alter insulin response.

# Eating Pattern Influences a Biological Rhythm in Gastric Responses to Food

An eating pattern of many small meals per day is useful in lowering blood cholesterol and, for non-insulin-dependent diabetics, in controlling blood glucose. Apparently, many people need to avoid unusually rapid absorption of sugars and of other nutrients after large meals. One may ask if the benefits of many small meals can be achieved with a practical number of meals per day. This question was approached with animal studies of gastric emptying, the rate-limiting step in digestion and absorption in the digestive tract. After test meals, gastric emptying occurred at different rates at different times of day, and a so-called gastric emptying response rhythm was defined.



This rhythm was controlled largely by the previous eating pattern of the rats. Early findings suggest that people may be able to avoid unduly rapid absorption of nutrients by adjusting the size and timing of a total of perhaps only three or four meals per day.

# Dietary Sucrose Induces Diabetic Retinopathy in Obese SHR/N-cp Rats

In collaboration with the National Eye Institute at NIH it was demonstrated that diabetic SHR/N-cp rats fed a high sucrose diet, develop retinal microangiopathies which are similar to those observed in human diabetic retinopathy. Alterations reported include proliferation of retinal capillaries and endothelial cells, pericyte loss, capillary dilation and varicose loop formation.

# Obesity and Diabetes Reduce Bone Growth in Rats With the SHR/N-cp Gene

Bone length and femur weight were reduced by both obesity and diabetes. The two effects were additive so that the obese diabetic had twice the bone reduction compared to the nondiabetic obese rat. The smaller bones were weaker, but had normal density and calcium content. Calcium retention and intestinal capacity to absorb calcium were both normal in either obese or diabetic rats. Calcium regulatory hormones (calcitonin, PTH) were both elevated in diabetic rats. The results indicate the existence of a major regulatory dysfunction of the hormones that regulate bone formation as a consequence of the presence of the N-cp gene.

## Trans Fatty Acids Have Deleterious Effects

Humans consume trans fatty acids through margarine and other hydrogenated oils. Those fatty acids have been shown to increase cholesterol level in humans. In monkeys, feeding 10% dietary fatty acids as trans fatty acids, we observed a decrease in the affinity of insulin receptor. However, there was no change in membrane fluidity which is normally increased by unsaturated fatty acids, and an increase in insulin receptor number. Our data show that dietary trans fatty acids are incorporated in erythrocyte membranes, and have the same effects on insulin receptor and membrane fluidity as seen with saturated fatty acids. The intake of trans fatty acids by humans, especially diabetic subjects, should be restricted. A study in humans is currently underway in collaboration with the Lipid Nutrition Laboratory, BHNRC and the Food and Drug Adminstration.

# Dietary Fat and Menstrual Cycle Affect Plasma Opiates in Humans

In humans, type of dietary fat as well as level of fat in the diet alters hormones such as insulin, glucagon, cortisol, dehydroepiandrosterone-sulphate, somatomedin-C and growth hormone. We have developed a new method which is simple and rapid for the simultaneous measurement of three opiates from small volume of human blood. Using this new method, we have demonstrated that plasma opiates are altered by the amount of fat in the diet, level of unsaturation of dietary fat and the menstrual cycle changes in premenopausal women.

# Atrial Natriuretic Peptide May Play a Role in the Cardiac Rupture in Copper Deficiency

Atria are now recognized as endocrine organs. They secrete atrial natriuretic peptides (ANP) which play a role in diuresis, natriuresis and hypertension. We have demonstrated sexual differences in plasma and atrial levels of ANP in copper deficient male and female rats and have postulated that an increase in plasma ANP which precedes



death from cardiac rupture may be responsible. We have further demonstrated that gonadal sex hormones are not involved in the sexual dimorphism of cardiac rupture and postulated that pituitary sex hormones may be involved. We are currently testing this hypothesis using hypophysectomized animals.



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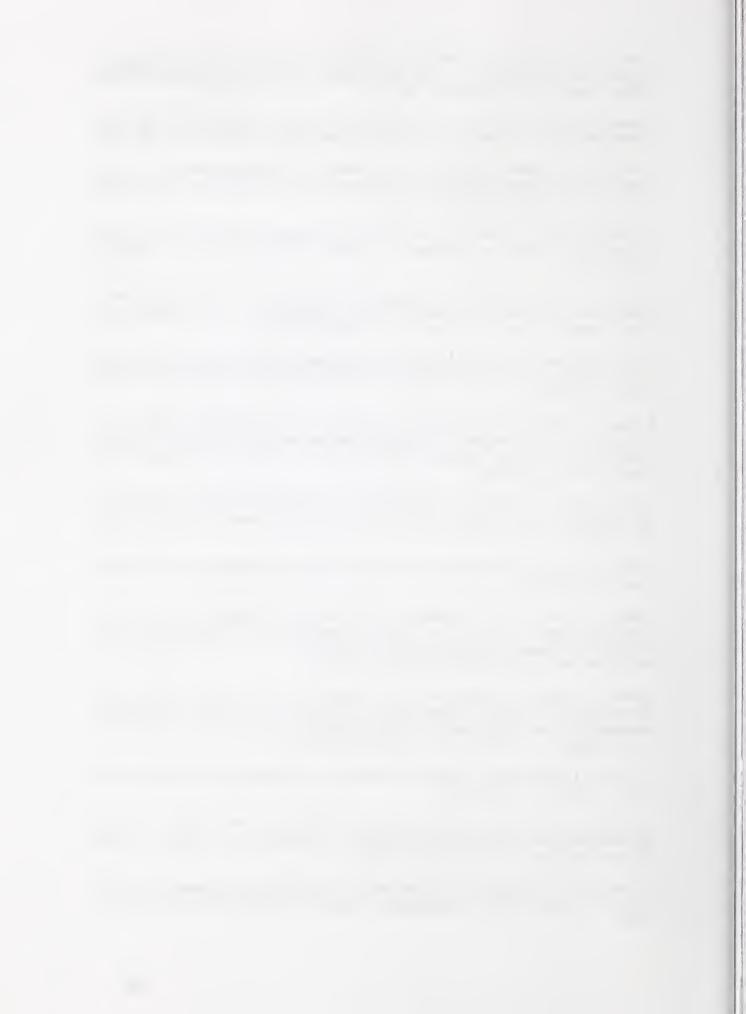
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## Energy and Protein Nutrition Laboratory Beltsville Human Nutrition Research Center Beltsville, Maryland 20705

Mission: To determine human energy and protein requirements as influenced by diet, activity and environment. To identify, quantify, and characterize the physiological processes involved in total energy expenditure. To identify the interactions among and the factors which control the composition of the body, energy expenditure and the source and amounts of nutrients consumed as it relates to the maintenance of desired body weight. To improve methods to predict energy values of foods from chemical or physical measurements. To characterize the metabolic responses to proteins including amino acid availability. To develop methods to indirectly assess the body composition and the energy expenditure of human subjects.

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Dr. James L. Seale Research Biomedical Engineer Room 211B, Building 308 Beltsville, Maryland 20705 301/504-8127 Provides leadership to the laboratory. Development of calorimetry facilities. Adequacy of Atwater factors in human diets. Effects of diet composition on energy use. Sources of variation in energy expenditure of humans.

Research focuses on the interactions of body composition, nutrient intake and energy expenditure. Utilizes stable isotopically labelled substrates to study the intermediary metabolism of protein, fat, and carbohydrate. Studies body composition changes during weight loss and in ethnic groups.

Studies the effects of varying nutrient intake on energy metabolism in normal and hyperinsulinemic individuals. Determines the physiological bases of variation among individuals in resting energy expenditure.

Effects of diet composition, plane of nutrition and environmental factors on the regulation of substrate utilization and energy balance in humans.

Development of direct and indirect calorimetry systems. Influence of physical activity, fitness level and environmental factors and on energy expenditure. Development of stable isotope method for estimating energy expenditure on free living populations.

#### RECENT RESEARCH ACCOMPLISHMENTS

## Energy Expenditure During the Menstrual Cycle

In studies using a canopy and bedside calorimetry system for indirect calorimetry on premenopausal women, there were no significant differences in resting energy expenditure (REE) measured at different times in the menstrual cycle. Thus changes in estradiol or progesterone had no detectable effect on REE. There was a 99% probability of detecting a difference of 7.85% in resting energy expenditure between measurements. However, similar studies in the BHNRC room-sized calorimeter showed that energy expenditure over a twenty-four hour period (24EE) differed significantly in relation to the menstrual cycle. In fact, energy expenditure during sleep (EEs) was highly influenced (P<0.0001) by the menstrual cycle. Results indicated that variation in 24EE reflects primarily changes in basal energy expenditure, as suggested by EEs values, and that this basal energy expenditure is increased about 6% when progesterone levels are elevated.

## Indirect Calorimetry on Ambulatory Subjects

An inexpensive, light, portable canopy was developed out of cellulose acetate. The canopy rests on a subject's shoulders, is sealed comfortably around the neck and allows casual movement. This apparatus, coupled with oxygen (conductivity) and carbon dioxide (infrared) analyzers is suitable for measuring diet-induced thermogenesis. Air enters at the bottom and is exhausted from the top of the canopy via a blower. Measured oxygen and carbon dioxide in the expired air is recorded and displayed on a personal computer.

## Energy Conservation Mechanisms by Men on a Reduced Calorie Diet

Short-term calorie deficiency diets are frequently utilized by people wishing rapid weight change. In this controlled study, healthy men were stabilized on their usual calorie intake for two weeks (3426 kcal/d) and then changed to a 50% reduced calorie content for the subsequent four weeks. Comparison of protein synthesis and glucose substrate cycling at the ends of the high and low calorie diet periods showed that an estimated 13% of the decrease in energy intake could be compensated for by decreased body weight (about 6%), decreased protein synthesis (about 6%) and reduced cycling (about 1%). The remaining 87% was met by the oxidation of body fat.

# <u>Metabolizable Energy Differences Between Low Fat, High Fiber and High Fat, Low Fiber Diets</u>

The digestibility of the energy and energy-containing nutrients was significantly lower in normal male subjects fed a low fat, high (64.8 g/day) fiber diet compared with a high fat, low (33.6 g/day) fiber diet. Fiber was supplied mainly from grain and cereal products. The metabolizable energy of these diets calculated from the U.S. food tables overestimated the measured metabolizable energy by a mean of 5%.

# Magnetic Resonance Imaging (MRI), a New Method for Assessing Changes in Adipose Tissue During Weight Reduction

MRI is a non-invasive technique for direct measurement of adipose tissue, muscle and bone. Thus this technique was used to determine the patterns of mobilization of adipose tissue during weight reduction in men on a 12 week calorie reduction diet. Adipose tissue volume was measured at the mid-point and termination of the study at



several body sites (arm, abdomen, hip, thigh, calf) and in addition % body fat and liver volume were measured. At the end of the diet period, there was a 13% reduction in weight, 30% reduction in percent fat, and a mean of 32% reduction in adipose tissue volume, which was not uniform at all measurement sites. Liver volume did not change.

# Changes in Energy Expenditure, Energy Balance and Body Fat Due to Calorie-restricted Diets

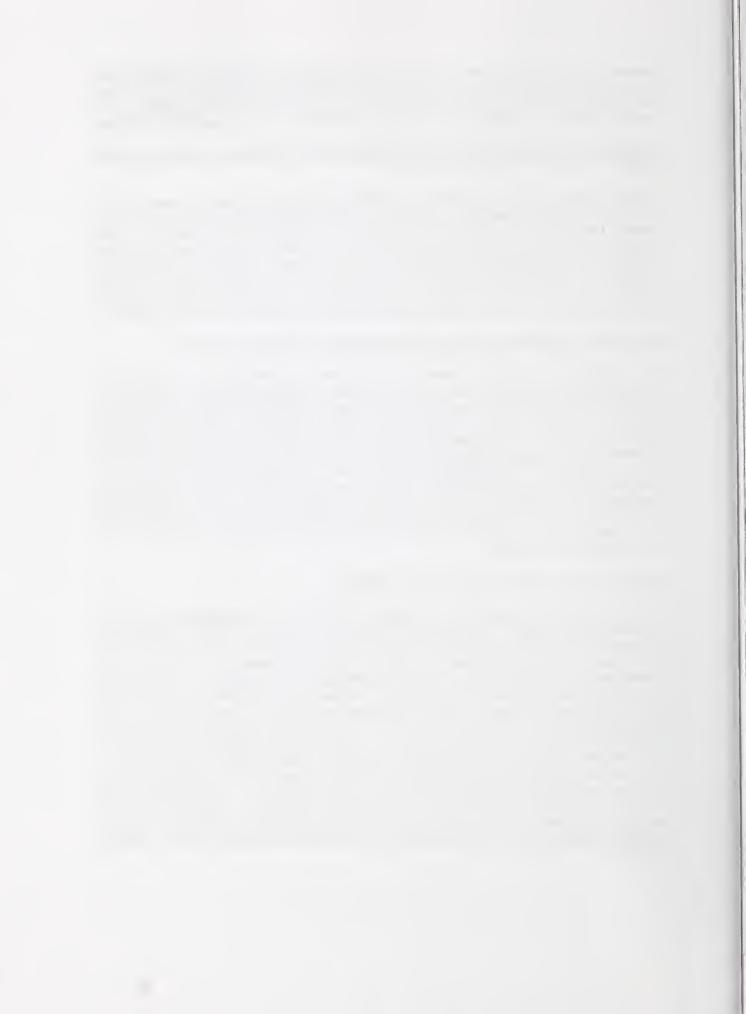
Normal male subjects were maintained on 3426 kcal/day diets containing either 20% or 40% calories from fat and then switched to 1763 kcal/d (50% for maintenance) for 4 weeks. During the reduced calorie period, there was a significant decrease in weight (96.6 to 91.5 kg); body fat (30.4 to 27.7%) and 24 hour energy expenditure as measured by indirect calorimetry (2902 to 2709 kcal/d). There was a significant diet effect on respiratory quotient (RQ): a decline of 0.84 to 0.82 on the high fat diet and 0.88 to 0.84 on the low fat diet. There was no effect on the type of fat diet during the reduction period on 24 hour energy expenditure or changes in body composition.

## Moderation of Carbohydrate and Lipid Metabolism With Dietary Amylose

The response of insulin, glucose, triglycerides and cholesterol to long-term consumption of high (70%) amylose starch products was determined in normal and hyperinsulinemic subjects. At the end of 12 weeks on diet, all subjects on the high amylose diet showed reduced postprandial insulin responses to a starch tolerance test when compared to the high amylopectin diet (P < 0.002). The reduction in insulin response was of a greater magnitude in hyperinsulinemic (P < 0.001), than in normal (P > 0.17), subjects. Fasting triglyceride levels were reduced (P < 0.0003) with time on diet, but fasting cholesterol levels remained unchanged. The results indicate that insulin levels of hyperinsulinemic men may be normalized following chronic consumption of high amylose containing foods. Increased starch consumption, particularly in the form of amylose, may help decrease blood lipids, a factor associated with atherogenesis in humans.

## Performance of BHNRC Room Sized Calorimeter

The Energy and Protein Nutrition Laboratory at BHNRC has two operational room sized calorimeter chambers. The dual direct/indirect calorimeter is 3.66m x 3.29m x 2.93m (volume 20m<sup>3</sup>) and is designed to measure (1) the actual heat emitted by a person within the room (direct calorimetry) and (2) heat production estimated from the oxygen consumed and carbon dioxide produced as a consequence of breathing the air circulated in the sealed chamber (indirect calorimetry). A water cooled gradient layer surrounds the chamber and provides the direct calorimetry measurement. Air composition and circulation rate are measured to determine the indirect calorimetry values. The second room sized chamber is a 3.41m x 3.41m x 2.93m (volume 21m<sup>3</sup>) indirect calorimeter. A computer automated data acquisition and control system is used to measure heat emission, air composition, temperatures, pressures and subject activity continuously in both chambers while the system is in operation. Performance tests indicate that the direct calorimeter determines heat emission with 97.3% and the indirect calorimeter measures heat production with 100.3% accuracy. To date approximately 800 24-hr energy expenditure measurements have been made on human subjects.



## Storage Efficiency of Excess Energy Intake

The efficiency of storage of metabolizable energy intake above maintenance was determined in individuals on high and low fat maintenance diets. Subjects were fed a high fat basal diet (HFB) or low fat (LFB) at maintenance. The diets were then additionally supplemented with either fat or carbohydrate at 25% above maintenance. Respiratory Quotient (RQ) averaged .81 and .89 for the HFB and LFB respectively. With the addition of fat the RQ declined slightly to .80 and .87 for HFB+fat and LFB+fat respectively. When carbohydrate was added the RQ increased to .86 and .95 for HFB+carbohydrate and LFB+carbohydrate. Examination of substrate oxidation demonstrates that the addition of carbohydrate had a fat sparing effect regardless of diet. Also, the addition of fat resulted in an increase in fat oxidation.

# Energy Expenditure Measured with Doubly Labeled Water in Free Living Adults Validated and Compared to Calorimetry

The doubly labeled water method as practiced at BHNRC was validated using the room calorimeter. The doubly labeled water method can be used to measure energy expenditure in free living adults over a one to two week period with minimal interference in the subjects' normal activities. For the purpose of validating this method nine subjects were given a dose of deuterium and oxygen-18 (nonradioactive, naturally occurring stable isotopes) and housed in the room calorimeter for a one week period. The doubly labeled water results were then compared to calorimetry results for that period. Free living energy expenditure was also determined for the one week period after the subjects left the chamber. Results indicate that the doubly labeled water method as practiced at BHNRC is accurate to 1.58% ± 1.72% (Mean ± SEM). Free living energy expenditure was 14% greater than the energy expenditure measured in the controlled restricted environment of a room sized calorimeter. These results compare favorably with the 15% greater free living energy expenditure determined in four adult men in a previous study.

# Energy Expenditure, Water Turnover, Metabolizable Energy, Nitrogen Balance and Protein Synthesis During 150 and 1000 ft Saturation Dives

Energy expenditure, water turnover rate, urinary output, metabolizable energy, nitrogen balance and protein synthesis were measured as part of a study to determine nutrient requirements for divers in deep saturation dives. Navy divers were studied for 10 to 14 days on the surface and during dry saturation dives in a helium-oxygen environment at depths of 150 and 1000 feet. Energy expenditure and water turnover were measured using the doubly labeled water method. Metabolizable energy and nitrogen balance were measured from analysis of duplicate meals, urine and feces. Protein synthesis was measured using ammonia and urea endpoint methods following a N15-glycine dose. Results indicate that energy expenditure increased by 12% at 150 feet and 14% at 1000 feet. There was no consistent change in water turnover. Metabolizable energy was not affected by the saturation environment and divers were in a positive nitrogen balance. Protein synthesis increased by 42% at 150 feet and 50% at 1000 feet. The helium-oxygen environment places a thermal stress on divers which accelerates metabolic rate at 150 feet. Additional respiratory and thermal stress may account for the higher metabolic rates observed at 1000 feet.

## Fat Patterning in Blacks

Anthropometry is frequently used as a rapid, inexpensive, non-invasive method of determining body composition. Generalized skinfold equations that were developed in



white populations were cross-validated in 90 blacks and 89 whites, with deuterium oxide dilution as the criterion reference method. It was determined that log-transformed, age and sex-specific equations developed by Durnin and Womersley successfully predicted body fat in our black population. In addition lower triceps-subscapular and thigh-subscapular ratios in black females and lower suprailiac-subscapular ratios in black males and females were found. It was therefore concluded that blacks may have more visceral and upper-body fat deposition than whites. This fat deposition pattern may be related to the increased incidence of hypertension and diabetes in blacks.

## Carbohydrate Restriction during Exercise

Sedentary, weight trained, and aerobically trained men were fed a high carbohydrate and moderately restricted carbohydrate diet. Ability to lift weights, time to fatigue, and exercise performance were not affected by carbohydrate restriction in any of the groups.



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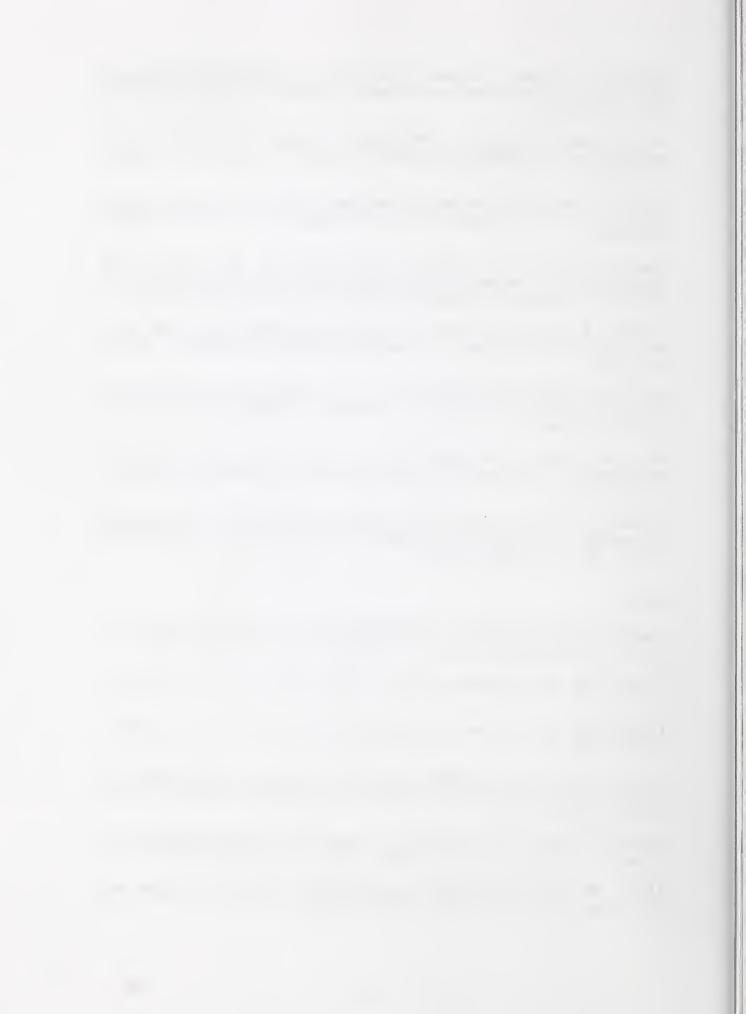
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### Lipid Nutrition Laboratory Beltsville Human Nutrition Research Center Beltsville, Maryland 20705

Mission: Determine effects of both kind and amount of dietary fat on metabolic and related physiological parameters in humans so that recommendations on optimal intake of fat and its constituent fatty acids are consistent with life-long maintenance of good health without adversely affecting quality of life.

Lack of knowledge of how other macro nutrients and non-nutritive components of the diet interact with lipids limits our ability to predict how different intake patterns will affect an individual. A major deficit in our understanding is how the relative amounts of the macro nutrients in the diet affect micro nutrient requirements for people of different ages. We also do not know how nutrient balance in foods or mixed diets affects bioavailability of micro nutrients.

The laboratory pursues this mission using free-living human volunteers and experimental animal models to: (1) investigate needs for essential fatty acids under different physiological conditions; (2) investigate the bioavailability of vitamins involved in lipid metabolism; and (3) investigate dietary lipid and cholesterol effects on physiological parameters related to good health.

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Dr. Elliott Berlin Research Chemist Room 109, Building 308 Beltsville, Maryland 20705 301/344-2297

Dr. Beverly A. Clevidence Research Nutritionist Room 115, Building 308 Beltsville, Maryland 20705 301/344-2430 Provides leadership to the laboratory. Current research includes investigating the role of lipids in the human diet in areas of critical importance to human health and well being.

Investigates the effects of changes in the dietary fatty acids including saturated monounsaturated, omega -3 and other polyunsaturated fatty acids on physical chemical properties of circulating plasma lipoproteins and cell and organelle membranes as related to the prevention of heart disease, cancer, and diabetes. Particularly interested in control of membrane fluidity and the relationship of fluidity to membrane physiology, e.g. receptor binding and receptor-ligand transport in membranes.

Determines the effects of dietary variables, particularly type and amount of fat, on plasma lipoprotein and apolipoprotein levels of healthy human subjects eating controlled diets. Investigates the influence of dietary variables on levels of alpha-tocopherol in plasma and distribution of this vitamin among lipoproteins.



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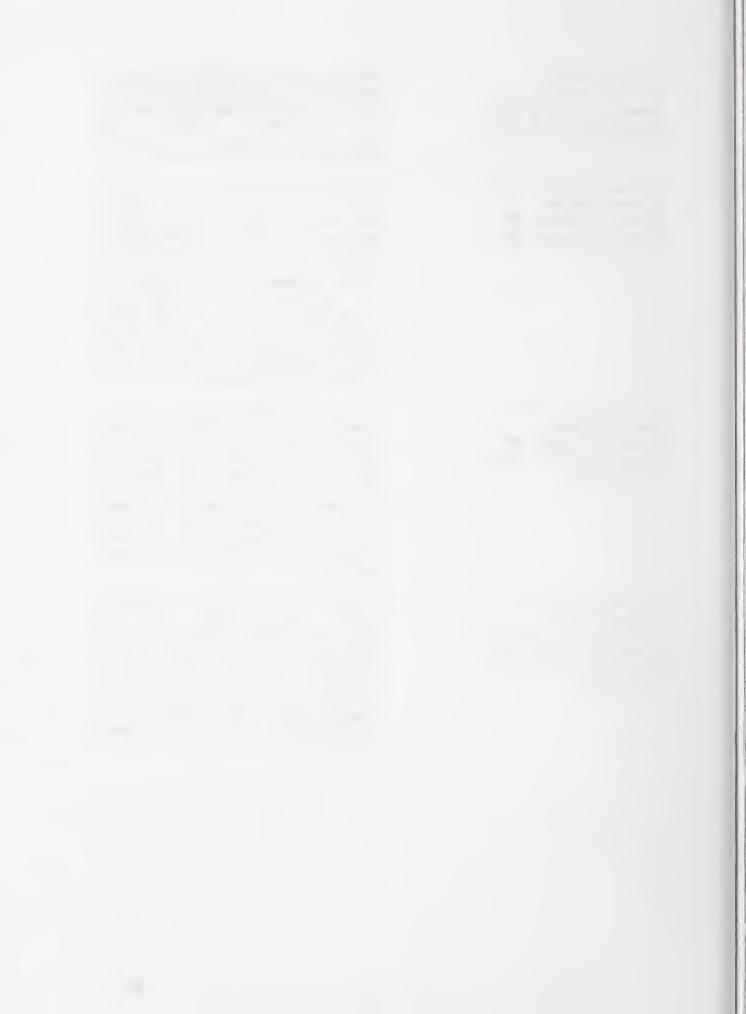
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Dr. John J. Podczasy Research Chemist (Post Doc with Dr. Schoene) Room 112, Building 308 Beltsville, Maryland 20705 301/344-2577 Investigates the biochemical mechanisms responsible for the physiological effects of dietary lipid modifications by studying the associated qualitative and quantitative changes of eicosanoid metabolism of essential fatty acids.

Conducts research on dietary lipids and their influence on human health, especially as related to dietary factors and the causation and/or prevention of cancer; and also the role of nutrition in delaying the process of aging, with special references to the susceptibility to carcinogenesis. Determines relationship of dietary fat and other nutrients to age-related disorders as reflected by changes in sterol and bile acid metabolism, fecal mutagenesis and glutathione sulfotransferase.

Investigates the relationship between essential fatty metabolism and prostaglandin production. Utilizes intact cells, especially blood platelets, to study the influences of diet on alterations in the production of these hormone-like lipids derived from essential fatty acids. Investigates the effects of dietary nutrients on cellular responses modulated by prostaglandins, e.g. platelet aggregation.

Conducts research on the effects of dietary fatty acids on the receptor-mediated generation of the intracellular ionized calcium signal. Correlates the calcium signal with other intracellular messengers to establish mechanisms of cellular transduction that result in specific physiological responses of blood platelets that can be modulated by dietary fat.



#### RECENT RESEARCH ACCOMPLISHMENTS

## Rats Fed Lithocholic Acid Show Increased HDL-Protein and HDL-Cholesterol

Rats fed low (20%) fat diets for 8 weeks containing 0.25% lithocholic acid had increased HDL-protein (106 vs. 88 mg%) and HDL-cholesterol (62 vs. 44 mg%); and a significant reduction in binding of human <sup>125</sup>I-HDL to hepatic membranes. These and associated data suggest that lithocholic acid may down-regulate hepatic HDL receptor activity and influence HDL metabolism thus elevating HDL-cholesterol.

### <u>Characterization of Extracellular Cholesterol-Rich Particles in Human</u> Atherosclerotic Lesions

Two types of cholesterol-containing particles were isolated from human atherosclerotic aortas and found to differ in that one is enriched with esterified and contains palmitate, oleate and linoleate as major fatty acids while the other is enriched with unesterified cholesterol and contains an additional fatty acid (stearate). The predominant phospholipid in both particles is sphingomyelin (56%).

## Bioavailability of Two Forms of Fish Fatty Acids Ingested by An Animal Model

Increases in dietary n-3 polyunsaturated fatty acids from fish oils have been recommended. In order to ingest considerable amounts of these oils, needed some individuals, a fish oil supplement may be needed. Odor and mouth feel of these oils are objectionable to some people. Using a rat animal model fed these fatty acids from menhaden fish, either in the form of free oil or the same oil contained in a digestible matrix microencapsulation, we showed that after one week on either of these controlled diets, bioavailability from either source to blood plately polyunsaturated acids is equal. Thus, microencapsulation is a promising delivery system for human studies.

## Quantitative Differences in Prostaglandins in Humans on High and Low-Fat Diets

A quantitative assay to estimate whole body synthesis of prostaglandins was developed and used to study differences attributed to dietary fat content. Men were fed either a high fat diet reflective of the current typical American diet 9.3% energy from polyunsaturated fatty acids) or a low-fat diet recommended to reduce risk from atheriothrombotic disorders and certain forms of cancers (6.6% energy from polyunsaturated fatty acids). Men on the low-fat diet showed an average of 14.2% reduction in daily prostaglandin output.

## Markers for Nutritonally-Related Aging Processes

Using biotechnological techniques, a quantitative procedure was developed for assessing mutagenicity changes in human stools that are responsive to dietary changes. Using this technique, fecal mutagenicity (marker for risk of colon cancer) was measured in subjects fed a typical high fat (40% energy) diet or a recommended low fat diet (20% energy). Our findings showed that lowering the fat content of diets decreased fecal mutagenicity, thereby clearly supporting the view that dietary fat could be a determinant of cancer risk.



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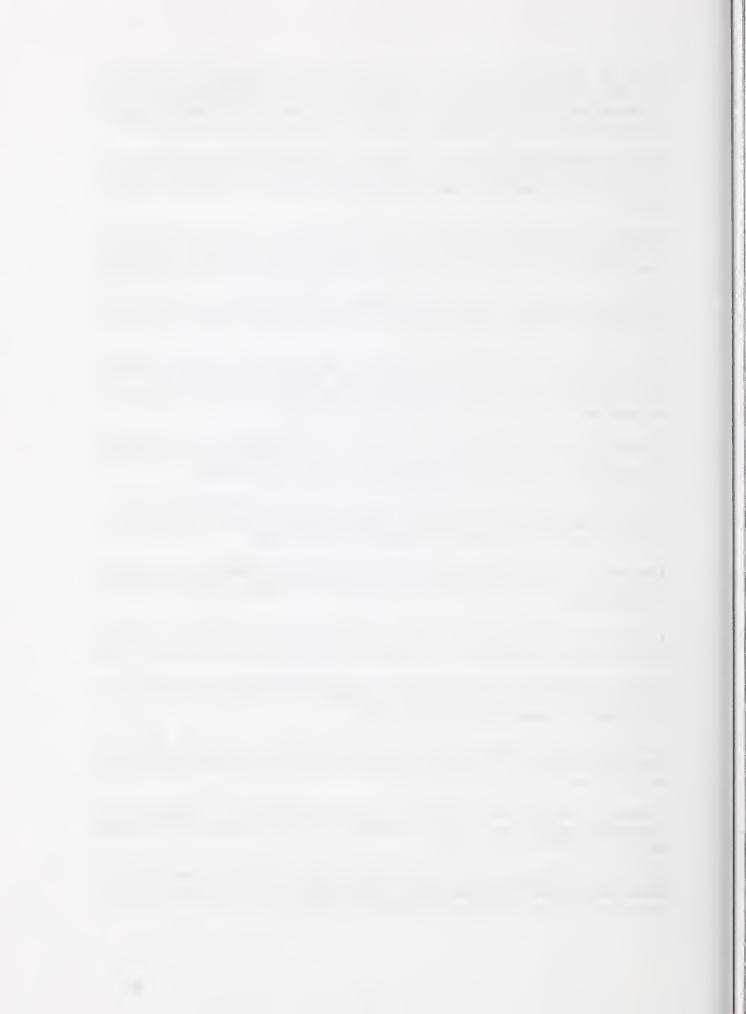
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# Nutrient Composition Laboratory Beltsville Human Nutrition Research Center Beltsville, Maryland 20705

Mission: The mission of the laboratory is to identify critical needs relative to the nutrient content of foods and to conduct research to meet these needs. To accomplish this mission, the research goals of the laboratory are: (1) to design and develop new and/or improved measurement systems for the analysis of nutrients and important other constituents in foods by conducting appropriate research in chemistry, biochemistry and biology, (2) to develop and utilize sound sampling techniques for the U.S. food supply to ensure that representative samples are analyzed for their nutrient content, (3) to transfer new technologies to industrial, academic and government laboratories both in the United States and world wide, (4) to analyze the nutrient content of foods with tested dependable assay techniques and supply results of these analyses to appropriate groups and agencies.

Dr. Gary R. Beecher Research Leader Supervisory Research Chemist Room 102, Building 161 Beltsville, Maryland 20705 301/504-8356

Dr. James M. Harnly Research Chemist Room 2, Building 161 Beltsville, Maryland 20705 301/504-8569

Joanne M. Holden Nutritionist Room 103, Building 161 Beltsville, Maryland 20705 301/504-8186 Coordinates research activities of the laboratory with research in other organizations including other USDA laboratories, NIH, FDA, universities and international organizations. Develops methodologies and measurement systems for the quantification of carotenoids and other phytochemicals in foods and diets that may be associated with nutritionally related disorders.

Develops multielement atomic absorption and emission instrumentation and methods for the determination of trace metals in micro samples. Employs graphite furnace atomizers, double furnace atomizers, solid state array detectors, high intensity xenen arc lamps, and glow discharges in developing new instrumental approaches.

Designs, develops, and implements statistically based sampling strategies which serve as the basis for selection of food samples to be analyzed. Develops expert systems for the evaluation of nutrient composition data. Serves on an international committee concerning the development of standardized food description. Coordinates food sample preparation and the dissemination of nutrient composition data.



Frederick Khachik Research Chemist (Research associate with Dr. Beecher) Room 104, Building 161 Beltsville, MD 20705 301/504-8830

Dr. Betty W. Li Research Chemist Room 105, Building 161 Beltsville, Maryland 20705 301/504-8466

Dr. Nancy J. Miller-Ihli Research Chemist Room 1, Building 161 Beltsville, Maryland 20705 301/504-8252

Dr. Raymond H. Thompson, Jr. Research Chemist Room 203B, Building 161 Beltsville, Maryland 20705 301/504-8789 Isolates and elucidates the chemical structure of carotenoids in fruits, vegetables, and human plasma by various spectroscopic techniques and organic synthesis. Develops liquid chromatographic techniques for purification, separation, and quantification of a broad spectrum of carotenoids from natural products and human plasma.

Develops accurate, high volume methods for carbohydrate analyses; especially sugars and starch determinations using gas-liquid and high-performance liquid chromatographic techniques. Develops reliable methods for the analysis of total dietary fiber by simplifying existing enzymatic/gravimetric procedures. Serves as an associate referee for total dietary fiber methods. Improving conditions for hydrolysis of polysaccharides, using microwave digestion system.

Develops methods and instrumentation for trace element determinations focusing on atomic spectroscopic techniques. Utilizes graphite furnace atomic absorption spectrometry (GFAAS) to perform direct analysis of solid samples prepared as slurries. Develops sample preparation and presentation procedures for trace metal analysis of biological materials. Develops and characterizes food quality control materials. Participates in the evaluation of analytical capabilities of commercial laboratories.

Development of improved methods to quantify lipids and lipid components in foods. Determination of fatty acids, cholesterol, plant sterols, and tocopherols using capillary gas-liquid chromatography (GLC). Application of multidimensional, capillary GLC (tandem column configurations with "valveless" stream splitting) for methods development in the quantification of cis-trans geometric and positional isomers of fatty acids. Development of improved lipid extraction methods.



Linda H. Tonucci Research Chemist (Post Doc with Dr. Beecher) Room 104, Building 161 Beltsville, MD 20705 301/504-8830

Dr. Joseph T. Vanderslice Research Chemist Room 202B, Building 161 Beltsville, Maryland 20705 301/504-9370

Dr. Wayne R. Wolf Research Chemist Room 4, Building 161 Beltsville, Maryland 20705 301/504-8927 Develops methods using supercritical fluids extract carotenoids from fruits, vegetables, and high fat foods. Investigates the qualitative and quantitative distribution of carotenoids in fruits and vegetables from a number of Pacific Islands. epidemiological studies have demonstrated a significant reduction in the incidence of several types of cancers among the populations of these Island Nations. In addition, identifies and quantifies carotenoids in tomato based products commonly consumed in the United States. These much needed data will be used in nutrient composition tables.

Determines vitamins in food and food extracts by high performance liquid chromatography; special emphasis is placed on developing extraction procedures which yield full vitamin recovery without destruction of any vitamin forms and which separate the vitamins from possible interfering compounds. Of particular interest at present are vitamin C and folacin.

Conducts research and coordinates joint programs with National Institute of Standards and other agencies for development of nutrient/food related Standard Reference Materials. Develops methodology and applications for determination of chemical species and bioavailability of trace elements utilizing chromatographic and microbiological techniques.



#### RECENT RESEARCH ACCOMPLISHMENTS

### Nonenzymatic-gravimetric Method for Total Dietary Fiber Analysis

Most gravimetric methods for total dietary fiber (TDF) determination require the complete removal of starch and the partial removal of protein with various combination of enzymes in buffers at different pH and at temperatures much above ambient condition. An enzymatic hydrolysis step is crucial in dietary fiber analysis of samples; such as cereals and legumes that contain appreciable amounts of starch. However, many vegetables and most fruits contain very little or no starch, and they are often eaten uncooked. It would be unnecessary to use high temperatures and enzymes on these types of samples. We were able to show that suspending the freeze-dried and ground samples in deionized water for 90 minutes at 37°C followed by the addition of 95% ethanol yields TDF values similar to those obtained from other published methods. An AOAC collaborative study is currently underway, results should be available for publication by November 1992.

### New Method for Vitamin C Analysis

A procedure for quantifying Vitamin C in foods and biological materials has been developed and successfully applied to a large variety of samples. The method distinguishes between compounds that exhibit vitamin activity and their isomers which have little or no activity. Since the latter are often used as antioxidants in foods, the method should prove useful for monitoring required by the new mandated labeling laws.

## New Source for Atomic Absorption Spectrometry

Developed a continuum source atomic absorption detection system with detection limits comparable to commercial instruments, but, with the potential for multielement determinations. Continuum sources offer light at all wavelengths for multielement determinations, but, are noisy and weak in the far UV (300 nm). By using a diode array detector, covering a small wavelength region around the absorption line, and pulsing the continuum source, detection limits are dramatically improved. Multielement determinations can be made by using multiple arrays in the focal planes.

#### New Atomic Emission Source

Established a low voltage arc inside a graphite furnace making the furnace an efficient multielement atomic emission source. Graphite furnaces are efficient atomizers, but do not furnish sufficient thermal energy to make sensitive emission sources. The low voltage arc is used for electrical excitation of the analyte atoms and produces electron excitation temperatures from 3000 to 4000°C. The result is an intense emission source with detection limits which are comparable to or better than graphite furnace atomic absorption, but, is readily adaptable to multielement determinations.

# Slurry Sampling for Graphite Furnace Atomic Absorption Spectrometry

The preparation of samples for analysis by atomic absorption spectrometry requires the use of strong acids and oxidizing agents. This is a long procedure and sample contamination is a concern. A method and associated instrumentation was developed whereby solid samples prepared as slurries, may be analyzed directly by graphite



furnace atomic absorption spectrometry (GFAAS). Slurries are prepared by diluting a small amount (5-50mg) of finely ground material with dilute acid and dispersing agent. Mixing is accomplished using an ultrasonic probe and this technology has been patented. Perkin-Elmer was granted an exclusive license and is currently marketing an autosampler accessory for slurry GFAAS Analyses. A wide variety of materials have been successfully determined using this approach which is especially well suited for environmental and health monitoring and product control.

## Microbiological Assay for Chemical Species of Selenium

This assay takes advantage of the presence in the bacteria <u>Escherichia coli</u> of formate dehydrogenase (FDH) enzymes that require selenium for synthesis. These FDH enzymes catalyze the formation of  $CO_2$  from formic acid, a sugar metabolite. By measuring  $CO_2$  production with a Bactec Infrared Analyzer we have developed a microbiological assay with linear dose response curves extending over several orders of magnitude down to the picomoles/ml range for selenomethionine and selenite. We are approaching the problem of determining chemical species by use of mutant strains of <u>E</u>. coli and the differential response of FDH enzymes to anaerobic and aerobic growth conditions.

## Development of a Carotenoids Database for the Assessment of Carotenoids Intake

The consumption of several fruits and vegetables has been inversely associated with the incidence of specific cancers in humans. Scientific investigation of these relationships require high quality composition data for the important contributors of dietary carotenoids. An expert system has been developed to evaluate the quality of published values for five carotenoids in 120 fruits and vegetables. Acceptable data have been combined with recipe formulations in the USDA National Nutrient Data Bank Recipe File to produce a database of approximately 2300 simple and complex foods with values for five carotenoids, alpha-carotene, beta-carotene, lutein, lycopene and beta-cryptoxanthin.

# Simplified Methods for Cholesterol Analysis

Developed a simplified procedure for quantitative analysis of cholesterol in fluid milk products. Also conducted research leading to an updating and simplification of the AOAC method for determination of cholesterol in multicomponent foods.

# New Total Diet Standard Reference Material for Nutrient Determinations

Standard Reference Material (SRM) 1548 Total Diet was developed for use in evaluating the reliability of analytical methods used for the determination of nutrient constituents related to health and disease in mixed diets, individual foods and biological matrices. This freeze dried powder material was prepared from excess foods obtained from the U.S. Food and Drug Administration's Total Diet Study (FDA-TDS) and was composited to be representative of an adult diet in the United States. The Certificate of Analysis for SRM 1548 provides certified concentrations and uncertainties for fourteen elements (N, Cl, Na, K, P,S, Ca, Mg, Fe, Zn, Mn, Cu, Se and Cd) in addition to values for fat, ash, Kjeldahl N, dietary fiber, cholesterol and energy content. The SRM 1548 is available through the Standard Reference Materials Programs, National Institute of Standards and Technology, Gaithersburg, Maryland.



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## Vitamin and Mineral Nutrition Laboratory Beltsville Human Nutrition Research Center Beltsville, Maryland 20705

Mission: The mission of the Vitamin and Mineral Nutrition Laboratory focuses on four main areas; (1) determine human requirements and basic mechanisms of action for specific vitamins and minerals; (2) identify chemical forms and bioavailabilities of vitamins and minerals in foods; (3) develop analytical instrumentation and techniques for assessment of minerals and vitamins in human nutrition; (4) develop and integrate cost-effective cellular, molecular and immunological methods for evaluating micronutrient bioavailability, interactions and requirements.

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Dr. Orville A. Levander Research Chemist Room 220B, Building 307 Beltsville, Maryland 20705 301/504-8504 Provides leadership to the laboratory. Investigates metabolism of zinc, copper, carotenoids, and vitamin A in animal models and humans. Assess methods for determining nutritional status for trace elements and vitamin A and studies specific nutrient interactions, such as vitamin A and zinc.

Investigates the effects of dietary chromium on risk factors associated with maturity-onset diabetes and cardiovascular diseases. Determines the effects of various stresses, primarily diet and exercise, on trace metal metabolism. Develops methods to assess chromium status and determine its mechanism of action.

Investigates the role of nutrients on the immune system. Determine nutrient requirements of humans experiencing various stress conditions, for optimal immune function in the host defense system, resulting in improved health status.

Investigates the role of selenium and vitamin E in human nutrition as can be clarified through studies on the functions and biochemical mode of action of selenium and vitamin E and their interrelationships. Other studies on the physiological need for these nutrients aim at determining the requirements under different conditions of stress (for example, parasitic infection) and development of accurate methods for assessing their status in humans.



Dr. Eugene Morris Research Chemist Room 215, Building 307 Beltsville, Maryland 20705 301/504-8282

Dr. Claude Veillon Research Chemist Room 226A, Building 307 Beltsville, Maryland 20705 301/504-9010 Investigates the impact of bioavailability factors on human mineral nutriture. A specific problem addressed is the amount of phytic acid and other inositol phosphates in foods as eaten.

Studies metabolism of trace elements required for human nutrition and develops new, accurate and precise methodologies for determination of trace elements in biological materials. Special attention is given to development of methods for measuring trace element status and requirements in humans using stable isotopes.



### RECENT RESEARCH ACCOMPLISHMENTS

## Use of Caco-2 Cell Line to Assess Nutrient Bioavailability and Interactions

Although fructose consumption has increased markedly in the U.S., the mechanisms of absorption of this sugar have not been as well studied as glucose. To study this we used Caco-2 cells as a model, since cultures of this human cell line develop many of the morphological and biochemical characteristics of mature enterocytes. Carbon-14-fructose was converted to CO2, glycogen and lipid, although at a level of 25-75% lower than for C-14 glucose. Addition of glucose, galactose, mannose or 2-deoxyglucose to the medium with C-14-fructose decreased incorporation of the radiolabel into CO2 and lipid. Fructose uptake by postconfluent cultures was 20-50% that of glucose. In addition, fructose uptake was decreased by addition of either glucose, galactose, or 2-deoxyglucose to the medium. Thus, fructose can serve as a carbon and energy source for the cells, but other simple sugars may impair its uptake and metabolism.

# Relationship of Copper, Selenium and Vitamin E to Cardiomyopathy in The Young, Growing Pig

Copper, selenium and vitamin E are involved in protecting the body from oxidative damage. Fructose has been suggested to initiate oxidative damage and to exacerbate copper deficiency. Therefore, this eight week study's goals were to examine the interrelationship between carbohydrates and copper, selenium and vitamin E. Casein based diets contained either 50% fructose, glucose or starch and either 6 ppm or 0.6 ppm copper. Mortality was greater in the fructose and glucose groups than starch. Hepatic glutathione peroxidase was not affected by diet but superoxide dismutase was decreased by the marginal copper diet. Circulating and cardiac vitamin E were higher in pigs fed starch. Regardless of treatment, histologic lesions suggestive of oxidant damage were found in 23 of 40 animals. These data suggest that copper may not be the only nutrient related to abnormalities associated with this interrelationship.

# Lack of Effect of Dietary Copper on Vitamin A Status

Several vitamin-mineral interactions have been documented including zinc and vitamin A. Copper and zinc share several similarities as transitional elements and have been shown to interact competitively. Indeed, a recent publication suggested "that a copper-deficient diet may cause defective transport of vitamin A ...", as has been forwarded in the case of zinc deficiency. Therefore, we designed a study using an experimental model (rats) fed copper deficient or adequate diets and controlled for food intake and weight gain. The copper deficiency was verified by several parameters. However, in spite of the marked copper deficiency, parameters of vitamin A status (serum and liver vitamin A concentrations) were not statistically different from the animals fed adequate dietary copper. Therefore, using this experimental model, we conclude that unlike zinc deficiency, inadequate dietary copper does not affect vitamin A metabolism. These data suggest that the vitamin A-zinc interaction is specific.

# Effect of Supplementation of Vitamin A and Zinc to Children of Marginal Status for These Nutrients

One-hundred thirty three children in Thailand, 6 to 13 years with marginal plasma vitamin A and zinc concentrations, participated in a double blind study. They were supplemented with either zinc (25 mg/day), vitamin A (1500 retinol equivalents/day), a combination of zinc and vitamin A or placebo for six months. Biochemical indices of



vitamin A and zinc status increased significantly. Zinc supplementation resulted in improvement of vision restoration time in dim light (dark adaptometry). Vitamin A and zinc synergistically normalized conjunctival epithelium as measured by conjunctival impression cytology. Both functional indices (dark adaptation and conjunctival impression cytology) showed significant correlation with plasma zinc and vitamin A, respectively. The data suggest that functional improvements of populations with suboptimal vitamin A and zinc nutriture can be accomplished by supplementation with less than two times the Recommended Dietary Allowance of these nutrients.

## Chromium Intake of Breast-fed Infants

Breast milk chromium content of 17 lactating women 60 days postpartum was analyzed to determine actual chromium intake of breast-fed infants. Dietary chromium intake of exclusively breast-fed infants was less than 2% of the minimum suggested safe and adequate daily intake. Present recommendations appear to be too high and need to be redefined.

## Insulin Stimulation is Altered by Albumin

Albumin is routinely added to the media when measuring insulin activity. However, our data demonstrate that while albumin activates insulin activity due to certain extracts such as those of brewer's yeast it inhibits activity of others, i.e., cinnamon. These data demonstrate that albumin effects need to be evaluated when assessing insulin activity.

## Exercise, Chromium and Trace Element Content of Tissues

Supplemental chromium caused an increase in copper, zinc and manganese concentrations in liver and pancreas of rats independent of training. Aerobic training led to increases in chromium, copper, iron, manganese and zinc in epididymal fat tissue. Copper concentrations of pancreas, kidney zinc and copper and zinc concentrations in gastrocnemius muscle were also higher. These data demonstrate that supplemental chromium and training lead to alter trace metal concentrations in tissues.

### Dietary Chromium Intake and Breast Milk Chromium Content

Dietary intake of chromium was shown to be independent of breast milk chromium content. Chromium concentrations in urine and serum also did not correlate with breast milk chromium concentration. However, there was a highly significant correlation of serum and urinary chromium. These data demonstrate that breast milk chromium is not closely regulated by dietary intake and that urinary chromium concentration is an accurate reflection of chromium circulating in blood.

# Chromium Improves Blood Glucose and Insulin Values

Chromium improved glucose tolerance and insulin values of chromium supplemented rats. Insulin responses were 43% lower and glucose 18% lower in chromium supplemented animals. These data demonstrate that dietary chromium improved glucose tolerance and decreases circulating insulin thereby enhancing tissue insulin sensitivity.



# Effects of Multiple Stressors During U.S. Army Ranger Training on Cell-Immune Functions

We sought to determine the effects of stressors, nutritional/physical, psychological, encountered during the 62-day Army Ranger Training Course on inflammatory and cellular immune functions. T-lymphocyte function in vitro blastogenesis and interleukin-2 production, is suppressed in trainees during the Ranger Training Course. The inflammatory/immunomodulating cytokine interleukin-6 is likewise reduced in the trainees during the Ranger Training Course. Our results demonstrate that the multiple stressors including food restriction, encountered during the Ranger Training Course suppress cellular immune functions in otherwise healthy, normal men.

# Effects of Micronutrient Supplementation on T-Lymphocyte Blastogenesis of Adults in Linxian County, China

We sought to determine the effects of micronutrient supplementation of adults with minerals and vitamins on cellular immune functions. Four hundred adults supplemented with selected micronutrients for five years were studied. Males supplemented with riboflavin, niacin, vitamin C and molybdenum showed significantly lower T-lymphocyte blastogenesis in vitro than males supplemented with selenium, vitamin E, beta-carotene, vitamin A and zinc. Males, but not females, supplemented with micronutrient combinations containing vitamin C and molybdenum showed lower T-lymphocyte blastogenesis than those not receiving them. Our results show that supplementation of males with combined vitamin C and molybdenum appear to have a suppressive effect on blastogenesis of T-lymphocytes.

# Aging, Diet, and Selenium Toxicity Alter Proton NMR Spectra of Rat Urine

Proton Nuclear Magnetic Resonance (PNMR) is a rapid method for the multi-component analysis of biofluids that requires no separation techniques. The non-selective nature of this procedure allows the detection and identification of a large number of low-molecular weight metabolites in urine with little or no sample pretreatment. We have found specific shifts in certain urinary metabolites as a result of aging, the type of diet fed, and selenium poisoning. Determination of urinary metabolite profiles by PNMR may allow development of selective tests for assessing a variety of physiologic states.

# No Evidence of Selenium Toxicity Found in a Human Population Residing in a Naturally Seleniferous Area

Adult men and women were recruited from South Dakota and Wyoming ranches where unusually high selenium intakes were suspected in order to determine whether high dietary selenium intake was associated with any adverse effects. Volunteers completed health questionnaires, underwent physical examinations, and provided blood samples for clinical assessment. Although dietary intakes more than 700 ug/day were reported, no physical findings characteristic of selenium toxicity were observed and no clinically significant changes in laboratory tests could be related to selenium exposure.

# Kinetic Modeling with Stable Isotopes Reveals Significant Differences in the Pharmacokineties of Organically-bound vs. Inorganic Selenium in Humans

Adult volunteers received a single oral dose of a stable isotope of 74-selenium in the form of selenomethionine. A kinetic model was developed to account simultaneously for the appearance and disappearance of the tracer in plasma, urine, and feces. The



whole body turnover of selenomethionine was considerably slower than that of selenite and this was ascribed to a substantial reutilization of selenium in this form. Such reutilization would have advantages for repleting persons of poor selenium status but might pose hazards to individuals exposed to excessive quantities of the element.

## Ground Flaxseed and Ethyl Linolenate Protect Vitamin E Deficient Mice Against Malaria

Several oils containing highly unsaturated fatty acids have marked antimalarial activity when fed to vitamin E deficient mice. Acceptance of such oils, however, could pose problems for field intervention trials. Incorporation of ground flaxseed into various baked goods could provide a convenient vehicle for the introduction of omega-3 fatty acids into Third World population groups as a means of malaria control. The fact that chemically pure linolenic acid exerted a significant antimalarial effect in tocopherol-deficient mice indicates that the antimalarial effect of fish oils and linseed oil can be accounted for solely on the basis of their omega-3 fatty acid content.

# Ascorbic Acid and Urinary Iron Excretion

Ascorbic acid enhances absorption of nonheme iron in single meal studies. However, only iron depleted individuals show an increase in iron stores, when measured by serum ferritin levels with continued consumption of ascorbic acid in meals. We investigated the question "could ascorbic acid increase urinary iron excretion and consequently negate the anticipated increase in body iron stores from enhanced nonheme iron absorption? Twelve ostensibly healthy adult men consumed approximately ten times the RDA of ascorbic acid with each of the two main meals daily for eight weeks. During the second week of ascorbic acid supplementation, mean daily urinary iron excretion was about 25% greater than for the week before supplementation, then tended to decrease from that maximum. Although urinary iron excretion tended to remain higher than pre-supplementation, the overall level of excretion for eight weeks was not statistically significant. We concluded that body iron homeostasis in the presence of high ascorbic acid consumption is not mediated through urinary iron excretion.

# Effect of Stress on the Biochemical Indices of Trace Minerals in Men

The objective of the study was to determine whether sustained physical and psychological stress would alter biochemical indices of trace mineral status in young, healthy men after they had undergone five weeks of prolonged physical training at submaximal intensities. Specifically, we were interested in determining the effects of five consecutive days of sustained physiological stress on biochemical indices of zinc, iron, copper, and selenium status in healthy, physically trained men. Subjects were 119 trainees from the US Navy Sea, Air, and Land (SEAL) training classes at the Basic Underwater Demolition School/SEAL Training Center in Coronado, California. Each was studied before and immediately after a period known as "Hell Week", a five-day period during which the trainees are subjected to extreme physical and psychological stress. The stress produced changes characteristic of an acute-phase response. Plasma zinc, iron, and selenium declines, while ferritin, ceruloplasmin, C-reactive protein, creatine kinase, and white blood cell counts rose. These changes were transient because all of the variables studied, except ferritin, returned to baseline values within one week after the sustained stress.



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